Remarks

The Office Action mailed January 28, 2003 has been received and reviewed. Claims 2-11, 23, 24, and 31-34 having been cancelled, claims 25 and 30 having been amended, and claims 35-50 having been added, the pending claims under examination are claims 25-30 and 35-50. Reconsideration and withdrawal of the rejections are respectfully requested.

As the Examiner requested, the specification has been amended at the paragraph beginning at page 1, immediately after CROSS-REFERENCE TO RELATED APPLICATIONS, to include the number of the issued patent.

The specification has been amended under Brief Description of the Drawings, at page 7, line 21, page 7, line 24, page 8, line 1, page 8, line 12, page 8, line 17, and page 8, line 23, to accurately reflect the figures themselves.

Support for the claim amendments is found throughout the specification. For example, support for amended claim 25 is found on page 21, lines 6-11 and page 11, lines 2-8 and lines 13-17, of the specification, and support for new claims 35-50 is found in original claim 25-30.

Information Disclosure Statements

The Examiner is thanked for considering the information disclosure statement submitted on May 25, 2001. However, the Examiner is requested to also consider the information disclosure statement submitted on August 23, 2002.

Objections to the Specification

The Examiner objected to the description of Figures, for not "accurately reflect[ing] the figures themselves." Following the Examiner's recommendation, the figure descriptions on page 7, line 21, page 7, line 24, page 8, line 1, page 8, line 12, and page 8, line 17, have been amended to include the appropriate reference to the subfigures.

The Examiner also objected to the description of Figure 9 on page 8 of the specification for not indicating which SEQ ID NO: is associated with which sequence shown in Figure 9. The description of Figure 9 has been amended to properly identify which SEQ ID NO:

is associated with each of the various sequences shown in Figure 9. Withdrawal of the objection to the specification is respectfully requested.

Objection to the Claims

The Examiner objected to claim 25 for containing a typographical error. Claim 25 has been amended to recite the proper spelling of 'activity.' Withdrawal of the objection to claim 25 is respectfully requested.

The 35 U.S.C. §112, Second Paragraph, Rejection

The Examiner rejected claims 25-30 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. This rejection is respectfully traversed.

Specifically, the Examiner asserted that the recitation "gene" in claim 25 was vague and confusing. Claim 25, as amended, no longer contains the recitation "gene."

The Examiner asserted that the recitation "the DNA fragment" in claim 25 lacks proper antecedent basis. Claim 25, as amended, no longer contains the recitation "DNA fragment."

The Examiner asserted that the recitation "the protein encoded by the gene" in claim 30 does not have proper antecedent basis in claim 25. Claim 30 has been amended to have proper antecedent basis in claim 25.

The Examiner asserted that the recitation "stringent conditions" in claim 25 is vague and indefinite. Claim 25 has been amended to recite "hybridizing under high stringency conditions to SEQ ID NO:1; wherein high stringency conditions are hybridization in a buffer containing 0.25 M Na_2HPO_4 (pH 7.4), 7% sodium dodecyl sulfate (SDS), 1% bovine serum albumin, 1.0 mM ethylene diamine tertaacetic acid (EDTA, pH 8) at 65° C, followed by washing 3x with 0.1% SDS and 0.1x SSC (0.1x SSC contains 0.015 M sodium chloride and 0.0015 M trisodium citrate, pH 7.0) at 65° C.)."

The Examiner asserted that the recitation "catalytic activity" in claim 25 is vague and indefinite. Claim 25 has been amended to recite "wherein an altered catalytic activity is selected from the group consisting of altered catalytic rate as quantified by k_{cat} and K_M, altered

substrate range, altered substrate preference, altered activity in aqueous solutions, altered stability in solvents, altered active temperature range, altered salt concentrations for enzymatic activity, altered pH for enzymatic activity, and improved activity in a soil environment."

For the reasons discussed above, withdrawal of the rejection of claims 25-30 under 35 U.S.C. §112, second paragraph, is respectfully requested.

The 35 U.S.C. §112, First Paragraph, Written Description Rejection

The Examiner rejected claims 25-30 under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This rejection is respectfully traversed.

Applicants respectfully submit that the assertion that the composition used in the method of claims 25-30 "comprises *any protein* . . . having an altered catalytic activity relative to the protein of SEQ ID NO:2" (p. 7 of the Office Action mailed January 28, 2003 (emphasis added)) is incorrect. The proteins of claims 25-29 are "encoded by a nucleic acid sequence capable of hybridizing under high stringency conditions to the sequence beginning at position 236 and ending at position 1655 of SEQ ID NO:1, . . . wherein there is at least one amino acid change in the protein encoded by the nucleic acid sequence as compared with SEQ ID NO:2." The protein of claim 30 "comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 5, 6 and 22-26." Thus, the proteins of claims 25-30 are described and claimed by sufficiently more detailed and relevant identifying characteristics than having an altered catalytic activity relative to the protein of SEQ ID NO:2.

It was also asserted that "[t]he specification, however, only provides those representative species . . . comprising a protein having the amino acid sequences of SEQ ID NO:5, 6 or 22-26. There is no disclosure of any particular structure to function/activity relationship in the disclosed species. . . . Given the lack of additional representative species . . . . Applicants have failed to sufficiently describe the claimed invention in such full, clear, concise, and exact terms that a skilled artisan would recognize Applicants were in possession of the claimed invention" (see p. 7-8 of the Office Action mailed January 28, 2003).

Applicants respectfully disagree with the Office's statement that "there is no disclosure of any particular structure to function/activity relationship in the disclosed species". This is not the proper standard to use when evaluating the claims for compliance with the written description requirement. According to the revised guidelines concerning compliance with the written description requirement of U.S.C. 112, first paragraph, it may be shown that "an invention is complete by disclosure of sufficiently detailed, relevant identifying characteristics which provide evidence that applicant was in possession of the claimed invention, i.e., complete or partial structure, other physical and/or chemical properties, functional characteristics when coupled with a known or disclosed correlation between function and structure, *or some combination of such characteristics*" (see MPEP 2163, pp. 2100-165 to 2100-166 (emphasis added)). Thus, adequate written description can be provided by some combination of complete or partial structure, other physical and/or chemical properties, or functional characteristics when coupled with a known or disclosed correlation between function and structure.

Applicants submit that the claims comply with the written description requirement. Specifically, claim 25 recites structural characteristics, i.e., "a protein encoded by a nucleic acid sequence capable of hybridizing under high stringency conditions to a sequence beginning at position 236 and ending at position 1655 of SEQ ID NO:1 . . . ." Claim 25 also recites functional characteristics, i.e., "wherein the protein has an altered catalytic activity as compared to the protein having the amino acid sequence of SEQ ID NO:2 wherein an altered catalytic activity is selected from the group consisting of altered catalytic rate as quantified by k_{cat} and K_M, altered substrate range, altered substrate preference, altered activity in aqueous solutions, altered stability in solvents, altered active temperature range, altered salt concentrations for enzymatic activity, altered pH for enzymatic activity, and improved activity in a soil environment."

Applicants also submit that a person of skill in the art would not expect substantial variation among species encompassed within the scope of the claims because the high stringency hybridization conditions set forth in the claim yield structurally similar nucleic acid sequences. Moreover, in view of this lack of substantial variation among species encompassed within the scope of the claims, the number of representative species required is low, and it is Applicants' position that the disclosure of 7 species, i.e., SEQ ID NO:5, 6 and 22-26, is a representative

numbers of species. Applicants respectfully submit that the claimed invention has been disclosed with sufficiently detailed, relevant identifying characteristics which provide evidence that applicant was in possession of the claimed invention and withdrawal of the rejection of claims 25-30 under 35 U.S.C. §112, first paragraph, is respectfully requested.

The 35 U.S.C. §102(a) Rejection

The Examiner rejected claims 25-30 under 35 U.S.C. §102 (a) as being anticipated by deSouza et al., *J. Bacteriology*, 1996 *78*: 4894-4900. Specifically, the Examiner asserted that deSouza et al. "teach the identification, isolation and cloning of the gene encoding atrazine chlorohydrolase from *Pseudomonas* sp. Strain ADP. . . . [and] further teach methods of treating a sample" that are the same as that claimed (see p.9 of the Office Action mailed January 28, 2003).

This rejection is respectfully traversed. According to MPEP § 2131 a "claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference."

Claims 25-29 are drawn to a method for treating a sample comprising "adding a composition to a sample comprising an *s*-triazine-containing compound; wherein the composition comprises a protein . . . wherein there is at least one amino acid change in the protein . . . as compared with SEQ ID NO:2; and wherein the protein has an altered catalytic activity as compared to the protein having the amino acid sequence of SEQ ID NO:2." In dependent claim 30, the protein is selected from the group consisting of SEQ ID NO:5, 6 and 22-26.

It is respectfully submitted that deSouza et al. teaches wild type atrazine chlorohydrolase from *Pseudomonas* sp. strain ADP. As shown in Figure 2 of deSouza et al., wild type atrazine chlorohydrolase from *Pseudomonas* sp. strain ADP has the amino acid sequence of SEQ ID NO:2. deSouza et al. does not teach the claimed polypeptide, "wherein there is at least one amino acid change in the protein . . . as compared with SEQ ID NO:2; and wherein the protein has an altered catalytic activity as compared to the protein having the amino acid sequence of SEQ ID NO:2." Neither does deSouza et al. teach a protein with the amino acid sequence of any of SEQ ID NO:5, 6 or 22-26.

Thus, Applicants respectfully submit that as deSouza et al. does not set forth each and every element of claims 25-30, deSouza et al. can not anticipate claims 25-30. Withdrawal of this rejection under 35 U.S.C. §102(a) is respectfully requested.

The 35 U.S.C. §102(b) Rejection

The Examiner rejected claims 25-27 and 30 under 35 U.S.C. 102 (b) as being anticipated by Mandelbaum et al., *App. Envir. Microbiol.* 1995; *61*:1451-1557. Specifically, the Examiner asserted that Mandelbaum et al. teach the isolation and characterization of a *Pseudomonas* sp. that mineralize the s-triazine herbicide atrazine and teach methods of treating a sample with the *Pseudomonas* sp. that are the same as that claimed (see p.9 of the Office Action mailed January 28, 2003). This rejection is respectfully traversed.

Claims 25-27 are drawn to a method for treating a sample comprising "adding a composition to a sample comprising an *s*-triazine-containing compound; wherein the composition comprises a protein . . . wherein there is at least one amino acid change in the protein . . . as compared with SEQ ID NO:2; and wherein the protein has an altered catalytic activity as compared to the protein having the amino acid sequence of SEQ ID NO:2." In dependent claim 30, the protein is selected from the group consisting of SEQ ID NO:5, 6 and 22-26.

It is respectfully submitted that Mandelbaum et al. teaches the isolation of a bacterium, a *Pseudomonas* sp. strain designated ADP, that is capable metabolizing atrazine at a very high concentration (see abstract). Mandelbaum et al. does not teach a polypeptide "wherein there is at least one amino acid change in the protein . . . as compared with SEQ ID NO:2; and wherein the protein has an altered catalytic activity as compared to the protein having the amino acid sequence of SEQ ID NO:2." Neither does Mandelbaum et al. teach a protein with the amino acid sequence of any of SEQ ID NO:5, 6 or 22-26. Thus, Applicants respectfully submit that as Mandelbaum et al. does not set forth each and every element of claims 25-27 and 30, Mandelbaum et al. can not anticipate claims 25-27 and 30. Withdrawal of this rejection under 35 U.S.C. §102(b) is respectfully requested.

Summary

It is respectfully submitted that the pending claims 25-30 and 35-50 are in condition for allowance and notification to that effect is respectfully requested. The Examiner is invited to contact Applicants' Representatives, at the below-listed telephone number, if it is believed that prosecution of this application may be assisted thereby.

Respectfully submitted for
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CERTIFICATE UNDER 37 CFR §1.8:
The undersigned hereby certifies that this paper is being transmitted by facsimile in accordance with 37 CFR §1.6(d) to the Patent and Trademark Office, addressed to Assistant Commissioner for Patents, Washington, D.C. 20231, on this 28th day of April, 2003, at 4:00 pm (Central Time).

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APPENDIX A - SPECIFICATION/CLAIM AMENDMENTS INCLUDING NOTATIONS TO INDICATE CHANGES MADE
Serial No.: 09/866,307
Docket No.: 110.00440102

Amendments to the following are indicated by underlining what has been added and bracketing what has been deleted.

In the Specification

Please replace the paragraph beginning at page 1, immediately after CROSS-REFERENCE TO RELATED APPLICATIONS, with the following rewritten paragraph.

The present application is a division of U.S. Patent Application No. 09/155,036, filed on 17 September 1998, issued as U.S. Patent No. 6,265,201, which in turn is a 371 filing of International Patent Application No. PCT/US98/00944, filed 16 January 1998, which in turn claims the benefit of U.S. Provisional Patent Application No. 60/035,404, filed 17 January 1997, all of which are hereby incorporated herein by reference in their entirety.

Please replace the paragraph beginning at page 7, line 21, with the following rewritten paragraph.

Figs. 1A-C. Nucleotide sequence alignment of wild type *atzA* (bottom sequence) from *Pseudomonas sp.* strain ADP and clone (A7) (SEQ ID NO:1 and SEQ ID NO:3). The boxed sequences indicate areas of nonidentity between the two nucleotide sequences.

Please replace the paragraph beginning at page 7, line 24, with the following rewritten paragraph.

Figs. 2A-C. Nucleotide sequence alignment of wild type *atzA* (bottom sequence) from *Pseudomonas sp.* strain ADP and clone (T7) (SEQ ID NO: 1 and SEQ ID NO:4). The boxed sequences indicate areas of nonidentity between the two nucleotide sequences.

Please replace the paragraph beginning at page 8, line 1, with the following rewritten paragraph.

Figs. 5A-D. Nucleotide sequence alignment of wild type *atzA* (SEQ ID NO:1, bottom sequence) from *Pseudomonas sp.* strain ADP and clone (A11). Fig. 5(a) provides the sequence from nucleic acids 11-543 (SEQ ID NO:7), Fig. 5(b) provides the sequence from nucleic acids 454-901 (SEQ ID NO:8), Fig. 5(c) provides the sequence from 1458-1851 (SEQ ID NO:9; N in this sequence indicates that this nucleotide has not been verified) and Fig. 5(d)

provides the sequence from nucleic acids 1125-1482 (SEQ ID NO:10) of clone A11. The boxed sequences indicate areas of nonidentity between the two nucleotide sequences. The "N" in these sequences refer to nucleic acids that are being verified. The four "C" nucleotides depicted above the top sequence in 5(a) and the eleven "G" nucleotides depicted above the top sequence in 5(b) indicate the correct nucleotide sequence of the top sequence.

Please replace the paragraph beginning at page 8, line 12, with the following rewritten paragraph.

Figs. 7A-B. [is a] are histograms illustrating the TERBUTHYLAZINE degradative ability of two homologs of this invention (T7= sample 3 and A7 = sample 4). Fig. 7(a) illustrates the % of TERBUTHYLAZINE remaining after exposure to AtzA or a homolog. Fig. 7(b) illustrates the relative amount of hydroxyterbuthylazine as a measure of TERBUTHYLAZINE degradation.

Please replace the paragraph beginning at page 8, line 17, with the following rewritten paragraph.

Figs. 8A-B. [is another set of] are histograms illustrating the terbutylazine degradative ability of three homologs A7, All, and T7. Figure 8(a) provides the % of TERBUTHYLAZINE remaining after a 15 minute exposure to the homolog in the presence or absence of the metals and additives of Samples 1-10. Figure 8(b) provides the relative amount of hydroxterbuthylazine in the presence or absence of the metals and compounds of Samples 1-10.

Please replace the paragraph beginning at page 8, line 23, with the following rewritten paragraph.

Fig. 9. is a comparison of PCR amplified fragments using two primers of the atrazine hydrochlorase gene from 6 different types of bacteria; *Pseudomonas* sp. Strain ADP (SEQ ID NO:16); *Ralstonia* strain M91-3 (SEQ ID NO:13); *Clavibacter* (*Clav.*) (SEQ ID NO:16); *Agrobacterium* strain J14(a) (SEQ ID NO:14); ND (an organism with no genus assigned) strain 38/38 (SEQ ID NO:15); and *Alcaligenese* strain SG1 (SEQ ID NO[S]:12[-16]).

In the Claims

For convenience, all pending claims are shown below.

25. [Amended] A method for treating a sample comprising an *s*-triazine-containing compound comprising the step of:

adding a composition to a sample comprising an *s*-triazine-containing compound,

wherein the composition comprises a protein encoded by a [gene having at least a portion of the] nucleic acid sequence [of the gene having at least 95% homology to the sequence beginning at position 236 and ending at position 1655 of SEQ ID NO:1,

wherein the gene is] capable of hybridizing under [stringent] high stringency conditions to the sequence beginning at position 236 and ending at position 1655 of SEQ ID NO:1,

wherein high stringency conditions are hybridization in a buffer containing 0.25 M Na_2HPO_4 (pH 7.4), 7% sodium dodecyl sulfate (SDS), 1% bovine serum albumin, 1.0 mM ethylene diamine tertaacetic acid (EDTA, pH 8) at 65° C, followed by washing 3x with 0.1% SDS and 0.1x SSC (0.1x SSC contains 0.015 M sodium chloride and 0.0015 M trisodium citrate, pH 7.0) at 65° C.),

wherein there is at least one amino acid change in the protein encoded by the [DNA fragment] nucleic acid sequence as compared with SEQ ID NO:2, and

wherein the protein has an altered catalytic activity [acitivity] as compared to the protein having the amino acid sequence of SEQ ID NO:2[;],

wherein an altered catalytic activity is selected from the group consisting of altered catalytic rate as quantified by k_{cat} and K_M, altered substrate range, altered substrate preference, altered activity in aqueous solutions, altered stability in solvents, altered active temperature range, altered salt concentrations for enzymatic activity, altered pH for enzymatic activity, and improved activity in a soil environment.

26. The method of Claim 25 wherein the composition comprises bacteria expressing the protein.

27. The method of Claim 25 wherein the *s*-triazine-containing compound is 2-chloro-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine.

28. The method of Claim 25 wherein the *s*-triazine-containing compound is 2-chloro-4-(ethylamino)-6-(tertiary butyl-amino)-1,3,5-triazine.

29. The method of Claim 25 wherein the *s*-triazine containing compound is (2,4,6-triamino-*s*-triazine).

30. [Amended] The method of Claim 25 wherein the protein [encoded by the gene is] comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 5, 6 and 22-26.

35. [New] The method of claim 25 wherein the sample is a water or soil sample.

36. [New] A method for treating a sample comprising an *s*-triazine-containing compound comprising the step of:

adding a composition to a sample comprising an *s*-triazine-containing compound,

wherein the composition comprises a protein encoded by a nucleic acid sequence having at least 95% homology to the sequence beginning at position 236 and ending at position 1655 of SEQ ID NO:1,

wherein there is at least one amino acid change in the protein encoded by the nucleic acid sequence as compared with SEQ ID NO:2, and

wherein the protein has an altered catalytic activity as compared to the protein having the amino acid sequence of SEQ ID NO:2,

wherein an altered catalytic activity is selected from the group consisting of altered catalytic rate as quantified by k_{cat} and K_M, altered substrate range, altered substrate preference, altered activity in aqueous solutions, altered stability in solvents, altered active temperature range, altered salt concentrations for enzymatic activity, altered pH for enzymatic activity, and improved activity in a soil environment.

37. [New] The method of claim 36 wherein the composition comprises bacteria expressing the protein.

38. [New] The method of claim 36 wherein the *s*-triazine -containing compound is 2-chloro-4-(ethylamino)-6-(isopropylamino)-1,3,5-triazine.

39. [New] The method of claim 36 wherein the *s*-triazine-containing compound is 2-chloro-4-(ethylamino)-6-(tertiary butyl-amino)-1,3,5-triazine.

40. [New] The method of claim 36 wherein the *s*-triazine containing compound is (2,4,6-triamino-*s*-triazine).

41. [New] The method of claim 36 wherein the protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 6 and 22-26.

42. [New] The method of claim 36 wherein the sample is a water or spoil sample.

43. [New] A method for treating a sample comprising an *s*-triazine-containing compound comprising the step of:

adding a composition to a sample comprising an *s*-triazine-containing compound,

wherein the composition comprises a protein encoded by a nucleic acid sequence capable of hybridizing under high stringency conditions to the sequence beginning at position 236 and ending at position 1655 of SEQ ID NO:1,

wherein high stringency conditions are hybridization in a buffer containing 0.25 M Na_2HPO_4 (pH 7.4), 7% sodium dodecyl sulfate (SDS), 1% bovine serum albumin, 1.0 mM ethylene diamine tertaacetic acid (EDTA, pH 8) at 65° C, followed by washing 3x with 0.1% SDS and 0.1x SSC (0.1x SSC contains 0.015 M sodium chloride and 0.0015 M trisodium citrate, pH 7.0) at 65° C.),

wherein there is at least one amino acid change in the protein encoded by the nucleic acid sequence as compared with SEQ ID NO:2, and

wherein the protein has an improved ability to degrade atrazine as compared to the protein having the amino acid sequence of SEQ ID NO:2.

44. [New] The method of claim 43 wherein the composition comprises bacteria expressing the protein.

45. [New] The method of claim 43 wherein the protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 6 and 22-26.

46. [New] The method of claim 43 wherein the sample is a water or spoil sample.

47. [New] A method for treating a sample comprising an *s*-triazine-containing compound comprising the step of:

adding a composition to a sample comprising an *s*-triazine-containing compound,

wherein the composition comprises a protein encoded by a nucleic acid sequence having at least 95% homology to the sequence beginning at position 236 and ending at position 1655 of SEQ ID NO:1,

wherein there is at least one amino acid change in the protein encoded by the nucleic acid sequence as compared with SEQ ID NO:2, and

wherein the protein has an improved ability to degrade atrazine as compared to the protein having the amino acid sequence of SEQ ID NO:2.

48. [New] The method of claim 47 wherein the composition comprises bacteria expressing the protein.

49. [New] The method of claim 47 wherein the protein comprises an amino acid sequence selected from the group consisting of SEQ ID NOS:5, 6 and 22-26.

50. [New] The method of claim 47 wherein the sample is a water or spoil sample.